A SYSTEM FOR COMPUTER-BASED RECONSTRUCTION OF 3-DIMENSIONAL STRUCTURES FROM SERIAL TISSUE SECTIONS: AN APPLICATION TO THE STUDY OF NORMAL AND NEOPLASTIC MAMMARY GLAND BIOLOGY.

Rodrigo Fernandez-Gonzalez, Arthur Jones, Enrique Garcia-Rodriguez, David Knowles, Damir Sudar and Carlos Ortiz de Solorzano

Life Sciences Division, Ernest Orlando Lawrence Berkeley National Laboratory, CA 94720

Tissue heterogeneity and three-dimensionality are generally neglected by most traditional analytical microscopy methods in Biology. These often disregard contextual information important for understanding most biological systems. In breast cancer, which is a tissue level disease, heterogeneity and three dimensionality are at the very base of cancer initiation and clonal progression. Thus, a three dimensional quantitative system that allows low resolution virtual reconstruction of the mammary gland from serial sections, followed by high resolution cell-level reconstruction and quantitative analysis of the ductal epithelium emerges as an essential tool in studying the disease. We present here a distributed microscopic imaging system which allows acquiring and registering low magnification (1 pixel = 5 μm) conventional (bright field or fluorescence) images of entire tissue sections; then it allows tracing (in 3D) the ducts of the mammary gland from adjacent sections, to create a 3D virtual reconstruction of the gland; finally it allows revisiting areas of interest for high resolution (1 pixel = 0.5 μm) imaging and automatic analysis. We illustrate the use of the system for the reconstruction of a small volume of breast tissue.

The system is based on a client-server architecture in which the client requests microscope operations and the server executes them. The communication between both sides is based on sockets, this being the standard communication method used for the Internet. The server runs on a computer physically connected to the microscope, and uses the low-level operations that the microscope driver provides. On the client side, the system presents a Java graphical user interface (GUI). Through this GUI, users can access some options directly linked to the microscope. Some of them are basic microscope operations (setting the objective lens, exposure time, fluorescence filter; focusing; acquiring single images, etc.), while some other are more complex ones, such as scanning multiple field-of-view areas or revisiting areas of interest from previously acquired images. The GUI also provides with a series of basic operations not directly linked to the microscope, such as creating cases (related sets of images), adding whole sections or areas of interest to a case, registering consecutive sections, marking and linking structures of interest on consecutive sections, etc. When any of the microscope-bound operations is requested, the client launches a new thread, which in time makes a request to the server, so that the user can keep working with the microscope non-related options in the main thread. This also guarantees that, in the case of a microscope failure or a socket error, the system will not die abruptly, as only the thread working on the microscope will be affected. Obviously, while one operation is being performed on the microscope no other operation is allowed on it. When the current operation finishes, the server returns the result of the operation (which could be an image or a simple acknowledgement message), and the client terminates the microscope thread and allows a new microscope operation to be requested.

The first step of the three dimensional reconstruction of a small volume of tissue is the low-resolution acquisition of the complete sections which form the tissue in which the volume is embedded (Fig 1). After registering these images, we proceed to mark the ducts in all of them. For this step we can use a wide variety of indicators such as points, lines or even shapes. Then the system reconstructs the tissue from the markings (Fig 2). This operation is fully performed at the client side of the system using the Java 3D API from Sun for the implementation. This keeps the client consistent, as it is fully implemented using Java. Finally, we can perform a gland-wide high-resolution analysis. This involves revisiting, acquiring and analyzing areas of interest, as defined by the user on the 3D reconstruction (e.g. reconstruct all primary ducts, branching areas, terminal buds, etc.) (Fig 1). This normally includes segmentation of the individual nuclei that form the epithelium of the ducts and detection of intranuclear elements such as genes (using Fluorescence In Situ Hybridization) or immunolabeled proteins.

The strengths of this system are mainly based upon the use of Java at the client side. This provides a universal tool that can be used from any web browser, on any platform, anywhere. The use of separate threads for microscope-linked and non-microscope-linked operations provides a flexible and robust application that allows

the sharing of images and resources from different research facilities given its distributed character. The system is soon to be applied to the study of the mechanisms of intraductal tumor spread and the differential expression of hormone receptors in normal versus neoplastic breast development. Future developments include automatic duct marking, to reduce human interaction.

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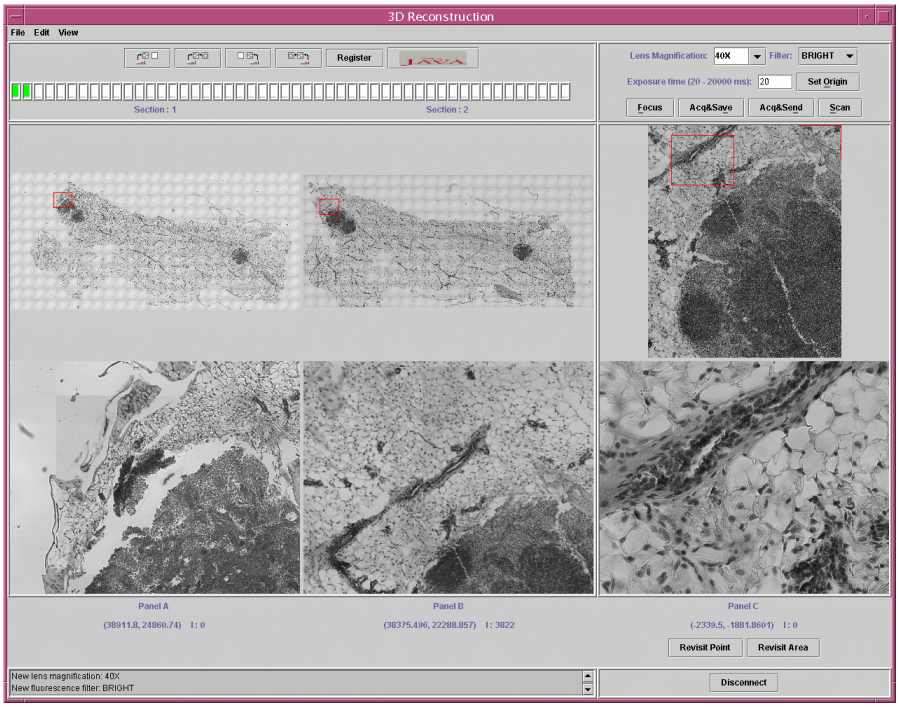

FIG 1

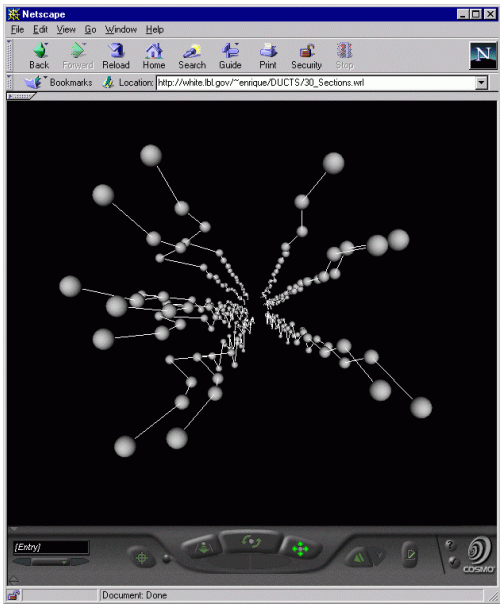

FIG 2

FIG 1. Snapshot of the Graphical User Interface. It shows two low-resolution (10X) consecutive sections (two left panels) and an magnified high-resolution (40X) image (rightmost panel) taken from an area of the image contained in the leftmost panel.

FIG 2. Three dimensional topological reconstruction of a small volume of the mammary gland of a mouse. The spheres correspond to ducts as interactively marked by the user on consecutive image

sections. The reconstruction is visualized using a web browser, and it is linked to the original images and slides to allow revisiting of areas of interest.